

ACCEPTANCE OF SIX ATYPICAL HOST SPECIES FOR OVIPOSITION  
BY *MICROPLITIS CROCEIPES* (HYMENOPTERA: BRACONIDAE)

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ABSTRACT

Comparisons were made of the acceptance for oviposition by the endoparasitoid, *Microplitis croceipes* (Cresson), of six atypical lepidopteran hosts with two typical hosts, the corn earworm, *Helicoverpa zea* (Boddie), and the tobacco budworm, *Heliothis virescens* (F.). The atypical hosts were: the fall armyworm, *Spodoptera frugiperda* (J.E. Smith); the beet armyworm, *S. exigua* (Hübner); the Indianmeal moth, *Plodia interpunctella* (Hübner); the cabbage looper, *Trichoplusia ni* (Hübner); the greater wax moth, *Galleria mellonella* (L.); and the diamondback moth, *Plutella xylostella* (L.). The acceptability of the atypical hosts for parasitoid oviposition was investigated after treatment of host larvae with corn earworm hemolymph, corn earworm frass, corn earworm frass plus corn earworm hemolymph, and also after exposure of host larvae to female parasitoids together with corn earworm larvae. Fall armyworm larvae were significantly more acceptable for oviposition by parasitoid females than the other atypical hosts, when untreated. The corn earworm frass plus hemolymph treatment had the most pronounced effect on the mean number of eggs laid/host across all six atypical species, followed by corn earworm hemolymph > corn earworm larvae > corn earworm frass.

KEY WORDS: *Microplitis croceipes*, Braconidae, endoparasitoid, host acceptance, atypical (and typical) host.

INTRODUCTION

In the southern United States, *Microplitis croceipes* (Cresson) is an important endoparasitoid of larvae of both the corn earworm, *Helicoverpa zea* (Boddie) (CEW) and the tobacco budworm, *Heliothis virescens* (F.) (TBW) (Snow et al., 1966; King et al., 1985; Powell and King, 1984). It is an effective parasitoid because it is specific for *Heliothis/Helicoverpa* spp., environmentally adaptable, tolerant of certain insecticide residues, and has a relatively high host search rate (Powell et al., 1986).

The lack of an inexpensive technology for mass propagating *M. croceipes* remains the main obstacle to evaluating this parasitoid in large-scale pilot tests in the field (King and Coleman, 1989). Currently, rearing *M. croceipes* on its typical host species, *H. zea* and *H. virescens*, for large-scale pilot tests, is cost prohibitive. Because the typical hosts are cannibalistic, the host

larvae must be reared individually, adding significantly to rearing costs (Powell and Hartley, 1987).

Recent studies have focused on developing an artificial rearing medium that would facilitate mass rearing of *M. croceipes* (Greany et al., 1989). However, so far, no hymenopterous larval endoparasitoid has been reared from egg to adult *in vitro* (Grenier et al., 1993), although *M. croceipes* has been reared to the first instar in tissue culture media conditioned with insect cell lines (Ferkovich et al., 1991; Ferkovich and Oberlander, 1991). An alternative approach would be to find an alternate host that *M. croceipes* females would accept for oviposition and on which the parasitoid could be reared more economically than on the typical host.

The host range of *M. croceipes* is generally limited to larvae of *H. zea* and *H. virescens* (Danks et al., 1979), although the parasitoid is capable of parasitizing *Heliothis subflexa* (Guenée) (Lewis, 1970; Krombein et al., 1979) and *H. phoxiphaga* Grote and Robinson (Bryan et al., 1969). Other larvae of host species that were exposed to *M. croceipes* but rejected for oviposition were the almond moth, *Cadra cautella* (Walker), the Indianmeal moth, *Plodia interpunctella* (Hübner) (IMM), and the lesser cornstalk borer, *Elasmopalpus lignosellus* (Zeller). Atypical hosts not readily attacked, but oviposited in by parasitoid females were the fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (FAW), beet armyworm, *S. exigua* (Hübner) (BAW), and the cabbage looper, *Trichoplusia ni* (Hübner) (CL) (Lewis, 1970). It has been suggested that some hosts are attacked not because they are preferred but because they are accessible in a particular habitat being searched and are acceptable (Vinson, 1976).

According to Doutt (1964) the final process in parasitoid host selection is host acceptance, and a number of factors are involved in this process. Physical stimuli such as size, shape, surface texture and chemical stimuli are important as well as host movement or vibrations in evoking oviposition (Vinson, 1977). In some cases, chemical odors alone or in combination with physical factors also play a role in host acceptance (Arthur, 1981). A number of chemicals stimulate ovipositor probing and thrusting (Jones et al., 1971; Schmidt, 1974; Henson et al., 1977). In *M. croceipes*, Lewis and Jones (1971) reported that contact with frass of the natural host, *H. zea*, resulted in antennation of the substrate and was important in locating and identifying suitable hosts. A similar response occurred upon treating the substrate with larval or pupal hemolymph from the typical hosts. The most active component isolated from *H. zea* frass was 13-methylhentriacontane (Jones et al., 1971; Jones, 1989). Although applying *H. zea* frass to an artificial oviposition substrate stimulates ovipositor probing, actual egg release depends on a kairomone found in the host's hemolymph (Tilden and Ferkovich, 1988; Eller et al., 1990; Heath et al., 1990). The hemolymph of the greater wax moth, *Galleria mellonella* (L.) (GWM) stimulated oviposition of *Itoplectis conquisitor* Say (Hegdekar and Arthur, 1973).

The use of alternate hosts for mass rearing has two requirements: first, the parasitoid female must accept the host for oviposition, and second, the parasitoid must successfully develop in the host. In this paper, we addressed the first requirement by studying the acceptability of six atypical host species for oviposition by *M. croceipes*. These atypical hosts are continually reared in our laboratory and were selected because they are less expensive to rear than *H. zea* and *H. virescens*. Also, since it is conceivable that an atypical host could potentially serve as a physiologically suitable host but not stimulate *M. croceipes* females to oviposit, we investigated means of inducing females to oviposit in hosts otherwise rejected by this parasitoid.

## MATERIALS AND METHODS

Two typical and six atypical hosts were examined. The typical hosts were CEW and TBW. The atypical hosts were FAW, BAW, IMM, CL, GWM, and the diamondback moth *Plutella xylostella* (L.) (DBM). All the host insects were reared according to Leppla et al., (1982). *Microplitis croceipes* was reared according to Ferkovich and Dillard (1986). Host larvae (third–fourth instar) of each test species that had not been exposed previously to parasitoids were placed in petri dishes with female wasps (5 female parasitoids/10 host larvae) for 1 h. Larvae of the two natural hosts and the six atypical hosts were treated prior to exposure to parasitoid females with: a) CEW hemolymph, b) CEW frass, c) CEW frass plus CEW hemolymph, or d) were exposed to parasitoid females together with CEW larvae (5 CEW larvae + 5 atypical host larvae) in the petri dishes. Hemolymph was collected from early fourth instar CEW larvae by clipping a proleg. Each host larva was then rolled in a drop of hemolymph and immediately exposed to the female parasitoids. Frass was smeared on the bottom of the petri dish, leaving a light coat of the material on the surface. The females used in the experiments were 4 days old, and had been exposed to parasitoid males but not to hosts. The host/parasitoid ratio was lower than that employed in our rearing procedure (Ferkovich and Dillard, 1986) using *H. zea* (10 host larvae/2 females). This lower ratio, which induced superparasitism in typical hosts, was used to insure oviposition in hosts unattractive to the parasitoid. Acceptance of the test hosts for oviposition by *M. croceipes* was evaluated 1 h after exposure to parasitoid females in terms of the number of eggs laid per host larva and percent parasitism. Host larvae were considered parasitized only if they contained one or more parasitoid eggs. All of the host larvae were dissected in a drop of IPL-52B tissue culture medium (GibcoBRL, Grand Island, NY) on a glass slide under a binocular microscope. The wasp eggs were easy to locate as they usually floated out of the host's hemocoel.

A weighted analysis of variance of the data was calculated using PROC GLM of the SAS computer package. Each treatment was replicated 6 times. A weight of one was assigned to the data in the treatment containing 5 CEW host larvae with 5 atypical host larvae and a weight of 2 to the other treatment groups containing 10 atypical host larvae. Separation of means was done using the Waller-Duncan procedure.

## RESULTS

### CEW and TBW

At the host/parasitoid ratio used in this study, the average number of eggs oviposited in the untreated typical hosts CEW and TBW was considerably higher than one egg/host (Table 1). In addition, none of the four treatments significantly affected oviposition into these species. Percent parasitism was 100% in untreated larvae of the typical hosts, CEW and TBW, as well as in each of the four treatments of CEW and TBW larvae (Table 2).

### FAW

Average number of eggs laid per host (Table 1) and percent parasitism of untreated FAW larvae (Table 2) were significantly lower than those of the two typical hosts, CEW and TBW, but higher than in the other five untreated atypical host species. In FAW, all four treatments increased oviposition and percent parasitism over the control larvae but none of the treatments

TABLE 1  
Effects of four treatments on the average number of eggs laid per host in two typical and six atypical host species parasitized by *M. croceipes*

Host species	Average number of parasitoid eggs/host <sup>1</sup>				
	Untreated host	Host CEW larvae	Host CEW hemolymph	Host CEW frass	Host CEW frass and hemolymph
Typical					
<i>H. zea</i> (CEW)	8.4 ± 1.6 <sup>a,A</sup>	8.3 ± 2.3 <sup>a,A</sup>	7.5 ± 1.4 <sup>a,A</sup>	7.7 ± 1.1 <sup>a,A</sup>	6.8 ± 0.8 <sup>a,A</sup>
<i>H. virescens</i> (TBW)	7.9 ± 2.1 <sup>a,A</sup>	7.9 ± 1.4 <sup>a,A</sup>	8.0 ± 2.6 <sup>a,A</sup>	7.8 ± 1.2 <sup>a,A</sup>	7.2 ± 0.6 <sup>a,A</sup>
Atypical					
<i>S. frugiperda</i> (FAW)	1.1 ± 0.5 <sup>b,B</sup>	3.1 ± 1.6 <sup>b,A</sup>	2.9 ± 1.8 <sup>b,A</sup>	3.1 ± 1.9 <sup>b,A</sup>	3.7 ± 0.8 <sup>b,A</sup>
<i>S. exigua</i> (BAW)	0.1 ± 0.2 <sup>c,C</sup>	1.2 ± 0.7 <sup>c,A,B</sup>	0.5 ± 0.6 <sup>c,B,C</sup>	0.9 ± 0.4 <sup>c,B,C</sup>	2.1 ± 1.5 <sup>c,d,A</sup>
<i>P. interpunctella</i> (IMM)	0.0 ± 0.0 <sup>c,B</sup>	0.7 ± 0.4 <sup>c,B</sup>	1.8 ± 0.9 <sup>b,c,A</sup>	0.4 ± 0.3 <sup>c,B</sup>	2.2 ± 1.2 <sup>c,A</sup>
<i>T. ni</i> (CL)	0.1 ± 0.2 <sup>c,B</sup>	0.4 ± 0.3 <sup>c,B</sup>	0.8 ± 0.8 <sup>c,A</sup>	0.4 ± 0.6 <sup>c,B</sup>	1.4 ± 0.4 <sup>c,d,A</sup>
<i>G. mellonella</i> (GWM)	0.1 ± 0.1 <sup>c,C</sup>	0.7 ± 0.6 <sup>c,B</sup>	1.1 ± 0.5 <sup>b,c,A,B</sup>	0.1 ± 0.1 <sup>c,C</sup>	1.2 ± 0.7 <sup>d,A</sup>
<i>P. xylostella</i> (DBM)	0.2 ± 0.4 <sup>c,B</sup>				2.2 ± 0.6 <sup>c,A</sup>

<sup>1</sup>All values are means ± SE. Within a column, means followed by the same lower case letter are not significantly different ( $p > 0.05$ ). Within a row, means followed by the same upper case letter are not significantly different ( $p > 0.05$ ).

TABLE 2  
Effects of four treatments on percent parasitism in two typical and six atypical host species parasitized by *M. croceipes*

Host species	Percent parasitism <sup>1</sup>				
	Untreated host	Host CEW larvae	Host CEW hemolymph	Host CEW frass	Host CEW frass and hemolymph
<b>Typical</b>					
<i>H. zea</i> (CEW)	100 ± 0 <sup>a,A</sup>	100 ± 0 <sup>a,A</sup>	100 ± 0 <sup>a,A</sup>	100 ± 0 <sup>a,A</sup>	100 ± 0 <sup>a,A</sup>
<i>H. virescens</i> (TBW)	100 ± 0 <sup>a,A</sup>	100 ± 0 <sup>a,A</sup>	100 ± 0 <sup>a,A</sup>	100 ± 0 <sup>a,A</sup>	100 ± 0 <sup>a,A</sup>
<b>Atypical</b>					
<i>S. frugiperda</i> (FAW)	58 ± 14 <sup>b,B</sup>	100 ± 0 <sup>a,A</sup>	87 ± 23 <sup>a,b,A</sup>	91 ± 11 <sup>a,A</sup>	97 ± 5 <sup>a,b,A</sup>
<i>S. exigua</i> (BAW)	9 ± 12 <sup>c,d,C</sup>	52 ± 21 <sup>b,B</sup>	42 ± 26 <sup>c,B</sup>	53 ± 18 <sup>b,B</sup>	85 ± 16 <sup>a,b,c,A</sup>
<i>P. interpunctella</i> (IMM)	0 ± 0 <sup>d,C</sup>	40 ± 13 <sup>b,B</sup>	75 ± 16 <sup>a,b,A</sup>	27 ± 20 <sup>c,B</sup>	77 ± 23 <sup>a,b,c,A</sup>
<i>T. ni</i> (CL)	10 ± 13 <sup>c,d,B</sup>	32 ± 29 <sup>b,B</sup>	37 ± 28 <sup>c,B</sup>	25 ± 27 <sup>c,B</sup>	80 ± 14 <sup>a,b,c,A</sup>
<i>G. mellonella</i> (GWM)	10 ± 13 <sup>c,d,B</sup>	49 ± 36 <sup>b,A</sup>	63 ± 29 <sup>b,c,A</sup>	10 ± 9 <sup>c,B</sup>	73 ± 28 <sup>c,A</sup>
<i>P. xylostella</i> (DBM)	15 ± 27 <sup>c,B</sup>				82 ± 10 <sup>a,b,c,A</sup>

<sup>1</sup>All values are means ± SE. Within a column, means followed by the same lower case letter are not significantly different ( $p > 0.05$ ). Within a row, means followed by the same upper case letter are not significantly different ( $p > 0.05$ ).

was significantly better than the other. Additionally, FAW larvae were also significantly more acceptable for oviposition compared with the other five atypical species in the CEW larvae, frass, and frass + hemolymph treatments, but not consistently in the CEW hemolymph-alone treatment. In FAW, all four treatments resulted in a high rate of parasitism, ranging from 87% in the hemolymph treatment to 100% in the CEW larval treatment.

#### **BAW, IMM, CL, GWM, and DBM**

*M. croceipes* females oviposited 0.1 egg/host in untreated larvae of BAW, CL and GWM, 0.2 egg/host in DBM, and none in IMM. These rates of oviposition are significantly lower than in the frass + hemolymph treatment (Table 1). Percent parasitism in untreated hosts was 0% in IMM, and ranged from 9% to 15% in BAW, CL, GWM and DBM. These rates of parasitism are significantly lower than in the frass + hemolymph treatment, which ranged from 73% in GWM to 85% in BAW (Table 2). The hemolymph-alone treatment stimulated attack of IMM, CL and GWM relative to the untreated larvae (Table 1). The CEW larvae treatment induced significant oviposition in BAW and GWM.

#### **DISCUSSION**

Generally, *M. croceipes* females oviposit only one egg per host (Lewis, 1970; personal observations), and one parasitoid develops in each host. Therefore, in these studies we considered successful parasitism to occur when an average of one or more eggs/host were oviposited. Based on the results presented in Table 1, only the FAW was considered to be acceptable by *M. croceipes* without having to treat the larvae to induce the parasitoid to oviposit. None of the other five atypical hosts were acceptable unless they were treated.

When taking as criterion the number of atypical hosts in which the amount of parasitoid eggs/host and the percent parasitism were significantly increased in comparison with the untreated hosts, we found that the most effective treatment was CEW frass + hemolymph, followed by CEW hemolymph > CEW larvae > CEW frass (Tables 1, 2). The CEW frass + hemolymph treatment was effective in all the six atypical hosts studied. Bartlett and Ball (1966) stated that host acceptance alone is not a reliable index of the overall suitability of the host for the parasitoid. Based on our results, all six atypical hosts in conjunction with the frass + hemolymph treatment may be used in studies on host suitability aimed toward identifying a cost-effective host for mass rearing *M. croceipes*. Lewis (1970) reported that females of *M. croceipes* could be prompted to sting 2 to 3 larvae of FAW, BAW, and CL, by repeatedly offering the hosts to parasitoid females, but that none of the parasitoids developed to the adult stage. In contrast, we found (Blumberg and Ferkovich, in preparation) that adult emergence of *M. croceipes* from FAW and GWM at 30°C was 13% and 21%, respectively, but only 4% and 3%, respectively, at 25°C. This indicates that studies on the manipulation of the rearing temperature of these two hosts may afford a high rate of production of *Microplitis* on these alternate hosts.

Hemolymph of CEW contains an oviposition-stimulating kairomone (Tilden and Ferkovich, 1988). That *M. croceipes* was stimulated to oviposit in the atypical hosts upon exposure to CEW frass alone and CEW larvae suggests that either the same compound found in the hemolymph or a different ovipositional cue was present in the CEW frass or emitted by the host larva. Schmidt (1974) concluded that chemicals that elicit acceptance behavior by

*Camponotus sonorensis* (Cameron), a parasitoid of CEW, were associated with larval frass, hemolymph, fat body, and labial or mandibular glands. Although frass of CEW is known to contain a host-seeking stimulant, 13-methylhentriacontane (Jones et al., 1971), it may also contain other chemicals in lower concentrations that stimulate oviposition by the parasitoid. That the combination of treatment with frass and hemolymph was more active in stimulating oviposition than either of these treatments alone suggests that possibly different chemical stimulants in each source complemented each other. The resultant high number of eggs oviposited per typical host larva in this study was probably due to the high ratio of parasitoid females/host (1:2) used in these experiments compared to that of 1 female/5 hosts employed in our usual rearing procedure for *M. croceipes* on *H. zea* (Ferkovich and Dillard, 1986). Moreover, it is evident from the high percentage of larvae being parasitized in each treatment that the females oviposited in a greater number of host larvae rather than in a few select ones. This may have been due to females marking the hosts they oviposited in with chemical cues to prevent other females from depositing eggs in the same host. This behavior has been observed by Debolt (1989) for the parasitoid *Leiophron uniformis* (Gahan) (Hymenoptera: Braconidae) and the host *Lygus lineolaris* (Palisot de Beauvois) (Heteroptera: Miridae). In addition, Debolt found that the host was capable of encapsulating many of the parasitoid eggs. He, therefore, suggested that the parasitoid was apparently using internal cues to avoid ovipositing in a host in which its eggs were likely to be destroyed by encapsulation. A similar situation probably occurred in the present study, since both percentage parasitism and the number of eggs per host were significantly lower in the atypical hosts which encapsulated many of the parasitoid eggs (Blumberg and Ferkovich, in preparation).

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